UNIVERSITI PUTRA MALAYSIA

SOME ASPECTS OF THE BREEDING BIOLOGY OF MACROBRACHIUM ROSENBERGII (De Man) WITH EMPHASIS ON ITS EGG INCUBATION AND LARVAL REARING

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SOME ASPECTS OF THE BREEDING BIOLOGY OF *MACROBRACHIUM ROSENBERGII* (De Man) WITH EMPHASIS ON ITS EGG INCUBATION AND LARVAL REARING

by

Kabir Ahmad bin Raffiq Ahmad

A thesis submitted in partial fulfilment of the requirements for the degree of Master of Science in the Faculty of Fisheries and Marine Science, Universiti Pertanian Malaysia.

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DEDICATION

This thesis is dedicated to my parents,my wife and children .

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Abstract

An abstract of the thesis presented to the Senate of Universiti Pertanian Malaysia in partial fulfilment of the requirements for the Degree of Master of Science.

SOME ASPECTS OF THE BREEDING BIOLOGY OF

MACROBRACHIUM ROSENBERGII (De Man)

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By

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Ovarian maturation, artificial incubation of eggs and larval rearing of M. rosenbergii (De Man) were studied. Female prawns were categorised into seven arbitrary developmental states based on the morphology of the gonads namely immatured females, matured females, pre-mating moult females, newly spawned females, females with orange colour eggs, females with grey colour eggs and post hatching females. The morphology and histology of the ovary for each developmental state was then studied. The mean oocyte size in the immature state was 47.0 $\pm$ 1.3 um and the oocyte size increased to a maximum of 402.0 $\pm$ 3.5 um at the pre-mating moult

state. After oviposition, the oocyte size diminished to 49.0 ± 1.4 um and then progressively increased . Two series of experiments were done to determine the optimum salinity and stocking density for artificial incubation of M.rosenbergii eggs. Newly laid eggs and 9 day old eggs were stocked at a density of 500/l and incubated at 0 ppt, 6 ppt and 12 ppt salinity respectively. The mean percentage of hatching after 18 days of incubation for the newly fertilised eggs were 0%, 3% and 40% as compared to 2%, 23% and 65% for 9 day old eggs. In the stocking density experiments, newly laid eggs were stocked at the rate of 1000/l, 1500/l and 2000/l at 12 ppt and their mean percentage of hatching were 37%, 33% and 16% respectively. Comparative dietary studies of larval culture were done using the closed recirculating system and the modified greenwater system. The larval diets tested were Diet 1 : microcapsules, Diet 2 : egg custard and Artemia and Diet 3 : microcapsules and Artemia. Larvae fed exclusively microcapsules did not survive beyond six days of larval culture in both the systems. There was no significant different ($P > 0.05$) in the production of juveniles between the two systems though higher production was obtained in the greenwater system. The mean number of juveniles obtained by Diet 2 using the greenwater system was 5.0/l. When Diet 3 was used and larval culture was done using the greenwater system, the mean number of juveniles obtained was 2.5/l. The production of juveniles using Diet 2 was significantly higher ($P<0.05$) than that of Diet 3 .

Abstrak

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia sebagai memenuhi sebahagian dari keperluan untuk ijazah Master Sains.

SOME ASPECTS OF THE BREEDING BIOLOGY OF

MACROBRACHIUM ROSENBERGII (De Man)

WITH EMPHASIS ON ITS EGG INCUBATION

AND LARVAL REARING

Oleh

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Kajian keatas proses kematangan ovari, pengeraman tiruan telur dan pemeliharaan larva bagi Macrobrachium rosenbergii (De Man) telah dijalankan. Udang-udang betina telah dikatogerikan kepada tujuh peringkat perkembangan yang berbeza berdasarkan kepada morfologi gonad iaitu peringkat betina yang belum matang, betina yang matang, betina yang bersalin kulit dan pra-mengawan, betina yang baru bertelur, betina dengan telur berwarna kuning, betina dengan telur berwarna kelabu dan betina selepas menetaskan telur. Kemudian morfologi dan histologi bagi ovari untuk setiap peringkat perkembangan dikaji. Min saiz oosit bagi peringkat

betina yang belum matang ialah 47.0 + 1.3 um dan saiz oosit telah bertambah ke peringkat maksimum iaitu 402.0 ± 3.5 um pada peringkat betina yang bersalin kulit dan pra-mengawan. Selepas oviposisi saiz oosit menjadi kecil kepada 49.0 ± 1.4 um dan kemudian saiznya semakin bertambah. Dua siri percubaan telah dijalankan untuk menentukan saliniti optimum dan kepadatan pelepasan bagi pengeraman telur M. rosenbergii secara tiruan. Telur-telur yang baru dan yang berusia 9 hari dieramkan secara berasingan pada kepadatan pelepasan 500/l pada saliniti 0 ppt, 6 ppt dan 12 ppt tiap-tiap satu. Peratus penetasan min yang diperolehi selepas pengeraman selama 18 hari bagi telur-telur yang baru disenyawakan ialah 0%,3% dan 40% berbanding dengan 2%, 23% dan 65% bagi telur-telur yang berusia 9 hari. Dalam percubaan kepadatan pelepasan, telur-telur yang baru telah dilepaskan pada kadar 1000/l, 1500/l dan 2000/l pada saliniti 12 ppt dan peratus penetasan min ialah 37%, 33% dan 16% bagi tiap-tiap satu. Kajian perbandingan makanan bagi pemeliharaan larva telah dijalankan dengan menggunakan sistem pusingan balik tertutup dengan satu unit penapis yang berasingan dan sistem air hijau yang telah diubahsuai. Makanan-makanan bagi larva yang telah diujikaji terdiri daripada makananan 1: mikrokapsul, 2: kastard telur dan Artemia, makanan 3: mikrokapsul dan Artemia. Kesemua larva yang diberi makan mikrokapsul sahaja didapati mati selepas enam hari dipelihara dalam kedua-dua sistem tersebut. Tiada perbezaan yang nyata ($P > 0.05$) dalam pengeluaran juwana diantara kedua-dua sistem walaupun pengeluaran juwana lebih tinggi dalam sistem air hijau. Bilangan min juwana yang diperolehi daripada

makanan 2 dengan menggunakan sistem air hijau ialah 5.0/l. Apabila makanan 3 digunakan dan pemeliharaan larva dijalankan dengan menggunakan sistem air hijau, bilangan min juwana yang diperolehi ialah 2.5/l. Pengeluaran juwana dengan menggunakan kastard telur dan Artemia didapati ternyata lebih tinggi ($P < 0.05$) berbanding dengan makanan 2.

CHAPTER I

GENERAL INTRODUCTION

The long legged Malaysian giant freshwater prawn, Macrobrachium rosenbergii (De Man) locally known as Udang Galah has since time immemorial been highly esteemed as food by the people of tropical countries in Asia, especially in the Far East. It is widely distributed in the South China Sea Region (Malecha, 1977). The genus Macrobrachium contains well over one hundred species which have been monographed for Southeast Asia and The Americas (Holthuis, 1952).

In the natural environment, M. rosenbergii juveniles and adults are reported to be omnivorous, feeding on various plant and animal materials including detritus, grass roots, insect larvae, small molluscs, crustaceans, flesh and offal of fish (Ling and Merican,1961; Ling,1962; Fujimura,1972; Machiolek, 1972). In captivity the juveniles of this prawn will accept a wide variety of food but the flesh of molluscs and crustacea seem to be their favourite and registered the best growth (Deshimaru and Shigeno, 1972; Forster and Beard,1973).

Ever since the major breakthrough by Ling (1962) on the breeding, hatching and larval rearing of this prawn, great interest has arisen to investigate the aquaculture potential of this species. Various techniques for rearing the larvae of this prawn have been developed in many parts of the world. The methods

of juvenile production have been documented by several workers (Fujimura , 1966 , 1967 , 1968 ,1972 ; Ling , 1969 ; Fujimura and Okamoto, 1970; Ling and Costello, 1976; Sandifer et al., 1976; Sandifer and Smith , 1977 ; AQUACOP , 1979) and in Malaysia the more recently published data are those by Ong et al. (1977), Cheah and Ang (1979), Aniello and Singh (1982), Lee (1982), Ong and Pang (1982), Ang and Cheah (1983), and Ong (1983).

Fujimura (1966) standardised the method of juvenile production by using "Greenwater" which consisted of unicelluar algae mainly Chlorella. Apart from the use of greenwater, some workers have experimented on static clearwater; closed recirculating clearwater and recirculating synthetic seawater, with varying degrees of success (Dugan et al.,1975; Smith et al., 1976; Hanson and Goodwin, 1977; Tansakul, 1983).

The larval stages of this prawn have been successfully reared using mullet (Mugil) eggs or dried chicken blood as primary food (Bardach et al., 1972). Chironomid larvae have been reported to have completely replaced Artemia in the diet of this prawn larvae in India (Hanson and Goodwin, 1977). Cyclops, Daphnia, Moina and other small planktonic crustacea, small pieces of fish, cooked fish ball, steamed hen's egg and fresh small fish eggs have been found to be good material as food for various larval stages (Ling, 1962). However low survival rates were obtained using Moina (Cheah, pers.comm., Aniello and Singh, 1982). Minamizawa and Morizane (1970) were able to rear M. rosenbergii, and M. formosense through all the larval stages using combinations of live Artemia salina nauplii, chopped fish

and chopped short neck clam.

In terms of stocking density in larval rearing, Sick and Beaty (1974) reported that the best stocking density for the highest growth and survival rate was 40 larvae per litre. However an intensive technique for freshwater prawn larval culture with densities of over 100 larvae per litre has been developed by Aquacop (1977, 1979). By using the same technique an experimental hatchery in Indonesia reported post larval production rates of 61 and 111 per litre (Haniah et al., 1982). With such progress in research there has been an apparent increase in the production of M. rosenbergii post larvae per unit volume of water.

Even though the breeding biology and larval rearing of this species has been well documented (Ling and Merican,1961; Ling 1962, 1969; Rao, 1965; Sandifer and Smith, 1979; Aquacop, 1977, 1979), there is no published information on its gonadal development in relation to maturation. Current hatchery practices involve the mother prawn incubating the eggs which is very energy consuming on the part of the prawn, thus an alternative method of hatching such as artificial incubation of the eggs should be investigated. Systems for mass production of juveniles of this prawn have been established. However, studies to reduce production costs and simplify the culture system by the use of artificial food to replace Artemia , an expensive food item, for the larval stages are still in progress. As a result of the problems highlighted above, the present experiments were designed with the following objectives in mind:

1. To study the morphology and histology of the ovaries of *M. rosenbergii* in relation to maturation;
2. To determine the feasibility of artificial incubation of *M. rosenbergii* eggs;
3. To evaluate the use of microcapsules as an artificial diet for the rearing of *M. rosenbergii* larvae.

CHAPTER II

THE MORPHOLOGY AND HISTOLOGY OF THE OVARIES OF MACROBRACHIUM ROSENBERGII

INTRODUCTION

Breeding behaviour in Macrobrachium rosenbergii have been excellently described by Rao (1965) and Ling (1969). Sexually mature males are able to mate at any time, while the females are ready to respond only after completing their pre-mating moult. It takes only a few minutes for the male and female prawns to get accustom to each other during the mating process. The male starts its courtship display by lifting its head, raising its body, waving its feelers and extending its long and powerful chelate-legs in an embracing gesture. This display continues for 10-20 minutes before the female is successfuly won over.

The male then holds the female between its long chelate-legs and at the same time actively cleans the ventral portion of her thoracic region with its other legs. It takes about 10-15 minutes to complete this clearing act and then follows the final mating act which lasts only a few seconds. The female is placed ventral side up while the male presses down from above. With a sudden vigorous vibration of the pleopods and trembling of the body the sperm is ejected and deposited in one gelatinous mass on the female's ventral median thoracic region.

It has been reported that the female of this species is able

to spawn three to four times in a year under natural conditions (Ling, 1969). However, there is no histological studies of its ovary to determine whether the animal is a multiple brooder.

Other species of Macrobrachium are known to be continuous breeders in captivity. Mauchline (1968, 1969) reported continuous oogenesis in different groups of Crustacea. Fish and Prece (1970) have also described continuous oogenesis in the amphipod genus Bathyporeia in which one set of embryos develop in the brood pouch while oogonia enlarge in the ovary. In Libinia emerginata the female crabs have a new egg mass in the brood chamber a short time after the zoea are released and are able to produce three to four consecutive broods (Hinch, 1968). Bauer (1976) reported that the shrimp Hectocarpus pictus is also a continuous breeder. While the female of this shrimp carries developing embryos, the ovary increases in size. Macrobrachium amazonicum is another species known to be a continuous breeder in captivity (Guest, 1979). Immediately after the female of M. amazonicum moults it mates and the eggs were attached to the pleopods the following day.

In order to determine the breeding pattern of M. rosenbergii the study described below was undertaken. This study was based on the morphological and histological examinations of the ovaries taken from different states of development of the females. The results of this study may be used as an index to show that M. rosenbergii is a multiple brooder.

MATERIALS AND METHODS

All the prawns for this study with the exception of the immatured ones were purchased from a fisherman at Sungai Linggi, Negeri Sembilan. Ninety-five female prawns were bought. The live prawns were packed under oxygen and transported to the Faculty of Fisheries and Marine Science, Universiti Pertanian Malaysia, Serdang, Selangor. The prawns were kept in two circular fibre glass tanks and were fed with frozen cockles once a day. This study was carried out from July-November, 1983.

The prawns were selected from the holding tanks and were arbitrarily classified into seven different states of development based on the condition of the females. The seven different states of the female classified in this study were as follows:

(a) Immatured Females

This group of prawns were reared from the larval stages in the hatchery and they were about four months old. The prawns were fed with minced cockles once a day. Ovaries of the prawns could not be seen externally through the carapace.

(b) Matured Females

Prawns in which the ovary occupied a large part of the cephalothorax and was generally visible through the carapace.

(c) Females At Pre-mating Moult.

For this group, the prawns were examined from time to time. This group consisted of females which had just undergone a

pre-mating moult and had a soft exoskeleton. The ovary was bright orange in colour and was clearly visible through the carapace.

(d) Females After Oviposition

Females which had just transferred the eggs from the ovary into the brood-pouch. The eggs were bright orange in colour.

(e) Berried Females with' Orange' Eggs.

Females which had incubated the eggs for a period of one week and the eggs were still orange in colour.

(f) Berried Females with 'Grey' Eggs.

Females which had already incubated the eggs for more than a week. Eye pigments were already formed in the eyes and the eggs looked grey in colour.

(g) Post Hatching Females

Berried females which had just released the larvae and the brood-pouch was devoid of eggs.

When the state of the females was determined, the prawns were sacrificed and their total length, post orbital length, body weight and the weight of the ovary were determined (Table I). For histological studies, ovaries taken from the females representing the seven different states, were cut into small pieces of about 1 cm before being introduced into 10 % buffered formalin. The ovaries were fixed for at least 12 hours before

being processed as outlined below:-

50% Alcohol	1 hour
70% Alcohol	1 hour
90% Alcohol	1 hour
Absolute Alcohol I	1 hour
Absolute Alcohol II	1 hour
Absolute Alcohol:	
Toluene 1:1	1 hour
Toluene I	1 hour
Toluene II	1 hour
Paraffin Wax I	3 hours
Paraffin Wax II	3 hours
Paraffin Wax III (vacuum)	1/2 hour

Sections were cut at 9 um thick using a rotary Microtome (Model 820, American Optical, USA) and then routinely stained using Mayer's Haematoxylin and alcoholic Eosin following Pantin's method (1969).

About 300 oocytes from each state of development of the females were measured using an ocular micrometer at a magnification of 100 X. The mean oocyte size at different states of development were then plotted (Figure 1).